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13. ABSTRACT *(Maximum 200 Words)*

Genomic imprinting is an epigenetic phenomenon in mammals that results in the differential expression of the paternally and maternally inherited alleles of a gene. Imprinted genes normally function to control embryonic growth and development. They also are involved in cancer because their functional haploid state makes them vulnerable to being either inactivated or overexpressed. The M6P/IGF2R has been shown to suppress cancer cell growth and is mutated in a number of human cancers, including those that develop in the lung, liver, colon and breast. These findings are consistent with the *M6P/IGF2R* functioning normally as a tumor suppressor. We have shown that *M6P/IGF2R* imprinting and receptor IGF2 binding evolved in an ancestor common to marsupials and eutherian mammals. Although *M6P/IGF2R* is imprinted in lower eutherian mammals, it is not imprinted in humans, as has been proposed in the literature. Our results with breast cancer and Wilms tumor suggest that a mutational event within intron 10 of the *M6P/IGF2R*, and not disregulation of imprinting, may have resulted in the aberrant monoallelic expression observed in some humans. Therefore, although our evidence strongly indicates that *M6P/IGF2R* functions as a tumor suppressor in lung, liver, colon and breast cancer, genomic imprinting at this locus is likely not involved in the etiology of tumorigenesis.

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INTRODUCTION

The *mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R)* gene encodes for a receptor that plays a critical role in regulating the bioavailability of extracellular proteolytic enzymes and growth factors known to be involved in carcinogenesis (*1,2*). Our recent findings indicate that the *M6P/IGF2R* also functions as a tumor suppressor gene in liver, breast, and lung cancer (*2,3,4,5*). We have determined that the frequency of monoallelic *M6P/IGF2R* expression in breast cancer patients is higher than that of age-matched controls. However, we have also demonstrated that the *M6P/IGF2R* is not imprinted in humans. Therefore, the observed monoallelic *M6P/IGF2R* expression in breast cancer is likely not the result of aberrant imprint regulation.

BODY

Genomic imprinting is a non-Mendelian, parent-of-origin specific, epigenetic form of gene regulation that results in monoallelic expression. The *M6P/IGF2R* is imprinted in both rats and mice, but imprinting at this locus is postulated to be a polymorphic trait in humans (For review see *2,4,6,7,8*). Because of this species difference in *M6P/IGF2R* imprinting, rodents would be predicted to be more sensitive than humans to cancer because only one allele would need to be mutated to inactivate its tumor suppressor function (*2,7*). Therefore, it is important to better understand the phenomenon of genomic imprinting, and its modification by both genotoxic and "non genotoxic" agents since rodents are used as surrogates for human cancer risk assessment (*8,9*).

The literature reports of *M6P/IGF2R* imprinting suggest that this gene may be polymorphically imprinted in humans, with some individuals expressing only the maternal allele and most other individuals expressing both parental alleles. Because of this uncertainty, and also due to our previous finding in Wilms tumor patients with monoallelic upstream *M6P/IGF2R* expression and biallelic downstream *M6P/IGF2R* expression, we wished to establish whether the *M6P/IGF2R* is subject to genomic imprinting. For some genes, imprinting can be age-dependent, with imprinted expression occurring only during early development. Therefore, to determine if the human *M6P/IGF2R* is imprinted, we utilized multiple organ tissues derived from 12 human conceptuses ranging in gestational age from 55 to 96 days. Using five polymorphisms within the *M6P/IGF2R* that were discovered in our laboratory (Killian, *et al.*, manuscript in preparation), all 12 conceptuses were shown to express *M6P/IGF2R* biallelically. This analysis included many individuals that were informative at multiple polymorphic sites, substantiating that the *M6P/IGF2R* is not subject to genomic imprinting during fetal development. We cannot, however, rule out the possibility that *M6P/IGF2R* imprinting occurs during embryonic development or in tissues not available for our analysis. However, recent experimental evidence from our laboratory, based on a detailed analysis of the evolution of imprinting of the *M6P/IGF2R* strongly supports the contention that the *M6P/IGF2R* is not imprinted in humans.

Genomic imprinting is postulated to have evolved because of a parent-offspring conflict to control fetal growth. This parental "tug-of-war" model predicts that only eutherian mammals would have imprinted genes because of the intrauterine development of their offspring. We tested this postulate by comparing *M6P/IGF2R* imprinting in monotremes (i.e. echidna and platypus), marsupials (i.e. opossum) and eutherian mammals (i.e. mouse, rat, pig, cow, bat, flying lemur, tree shrew, ringtail lemur and humans). Our findings demonstrate that *M6P/IGF2R* is not imprinted in the egg-laying platypus and echidna, whereas it is imprinted in the opossum (*10*). Thus, imprinting evolved in viviparous mammals over 100 million years ago; however, since the opossum lacks a fetal stage of development, invasive placentation and intrauterine fetal growth are not required for genomic imprinting to evolve. The *M6P/IGF2R* in both the monotremes and didelphid marsupials also lacks the differentially-methylated CpG island in intron 2 previously postulated to be mechanistically involved in imprint control in mice. This demonstrates the existence of alternative mechanisms of *M6P/IGF2R* imprint establishment and maintenance. Our results also indicate that monotremes and marsupials are not as closely related as predicted by the Marsupionta model; instead, they support the morphology-based Theria hypothesis of mammalian evolution.

We have also shown that although the *M6P/IGF2R* is imprinted in mice, rats, pigs, cows, and bats, imprinting at this locus was lost approximately 70 million years ago with the evolution of the higher mammalian orders: Dermoptera (e.g. flying lemurs), Scandentia (e.g. tree shrews), and Primates (e.g. ringtail lemurs and humans) (Killian *et al.*, manuscript in preparation). This finding provides compelling evidence that *M6P/IGF2R* imprinting is not a polymorphic trait in humans since convergent evolution of *M6P/IGF2R* imprinting would have had to have occurred in humans. This highly unlikely possibility is also supported by our inability to demonstrate *M6P/IGF2R* imprinting in either fetal or adult human tissues. The lack of *M6P/IGF2R* imprinting in humans has important ramifications in toxicology because it strongly indicates that although the *M6P/IGF2R* functions as a tumor suppressor in humans and rodents, rodents are at heightened susceptibility to tumor formation because of the imprinted status and consequent functionally haploid state of the *M6p/Igf2r*.

Key Research Accomplishments

- *M6P/IGF2R* is frequently mutated in human breast, liver and lung cancer suggesting it functions as a tumor suppressor gene.

- Monoallelic *M6P/IGF2R* 3' end gene expression was found in 2/32 (6.3%) of breast cancer patients, suggestive of a chromosomal deletion event or a posttranscriptional mechanism which results in the production of a single or truncated mRNA species.

- We have determined that in Wilms tumor patients, a truncated *M6P/IGF2R* transcript is produced from one allele and have further mapped the site of truncation to within intron 10 of the *M6P/IGF2R* gene.

- *M6P/IGF2R* is not imprinted in humans during fetal development.

- *M6P/IGF2R* imprinting (i.e. maternal expression) and receptor IGF2 binding evolved in an ancestor common to marsupials and eutherian mammals. The evolution of this parent-of-origin expression purportedly occurred because of a parental genetic conflict to control offspring growth and development.

- The evolutionary loss of imprinting of the *M6P/IGF2R* in higher mammals (Dermoptera, Scandentia, and Primates) approximately 70 million years ago strongly supports that the *M6P/IGF2R* is not imprinted in humans.

- The finding that the *M6P/IGF2R* is not imprinted in humans suggests that imprinting disregulation is not a contributing factor in the etiology of breast cancer and Wilms tumor.

Reportable Outcomes

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- Personnel active on this project : Susan K. Murphy, Ph.D., J. Keith Killian (graduate student), Catherine A. Nolan, Ph.D. and Andrew A. Wylie, Ph.D.

Conclusions

In conclusion, there is now compelling mutational and functional evidence that the *M6P/IGF2R* is a tumor suppressor that is frequently inactivated during the early stages of

human cancer formation. *M6P/IGF2R* loss of function not only provides cancer cells with an early growth advantage, but also confers enhanced resistance to radiotherapy treatment. However, the lack of *M6P/IGF2R* imprinting indicates that this gene does not confer susceptibility to cancer because of genomic imprinting. Imprinted genes do provide unique susceptibility loci for cancer and behavioral disorders. Consequently, identifying those genes that are imprinted and the epigenetic mechanisms by which their expression is controlled will greatly enhance our understanding of the molecular mechanisms underlying cancer susceptibility at imprinted gene loci. We will therefore continue our efforts to identify novel imprinted genes and their control mechanisms.

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